



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Serial No.: 09/328,975 )  
Filed: 6/9/99 )  
Group Art Unit: 1632 )

Examiner: Richard Schnizer

For: Charge Reversal of Polyion Complexes

**DECLARATION UNDER 37 C.F.R. §1.132**

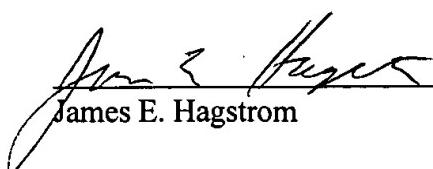
Assistant Commissioner for Patents  
Washington, DC 20231

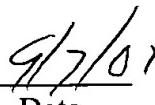
Dear Sir:

I, James E. Hagstrom, hereby declare as follows:

1. I am an inventor of the captioned application.
2. Applicants' process was conceived prior to the effective date of the Office Action prior art references.
3. We developed our recharging process with due diligence from conception to the filing of our application.
4. Photocopies of my personal laboratory notebook pages showing nucleic acids recharged with histone and then re-charged with liposomes dated March and June, 1994 accompany this Declaration.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

  
James E. Hagstrom

  
Date  
9/7/01

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3/28/

## RAT Myotube Transfection

↙ ALL DNA in pH 8.5 tris

- 1> 5 ug pBSRLUX 5ug/plate
- 2> DNA + Lipofectin 2.5λ/plate
- 3> DNA + Lipofectin 15λ/plate
- 4> DNA + Lipo (2.5λ/plate) + NLS-H1 (0.5μg/plate)<sup>(1,2)</sup>
- 5> DNA + Lipo (2.5λ/plate) + NLS-H1 (1.5μg/plate)<sup>(3,4)</sup>
- 6> DNA + Lipo (2.5λ/plate) + NLS-H1 (0.5μg/plate) + MAT-H1 (0.5μg/plate)<sup>(2,3)</sup><sub>(punc)</sub>
- 7> DNA + Lipo (2.5λ/plate) + NLS-H1 (0.5μg/plate) + MAT-H1 (1.5μg/plate)<sup>(4,2)</sup><sub>(CB rare)</sub>
- 8> DNA + Lipo (2.5λ/plate) + NLS-H1 (1.5μg/plate) + MAT-H1 (0.5μg/plate)<sup>(2,3)</sup><sub>(CB rare)</sub>
- 9> DNA + Lipo " " + NLS-H1 (1.5μg/plate) + MAT-H1 (1.5μg/plate)<sup>(3,4)</sup><sub>(CB rare)</sub>
- 10> DNA + Lipo " " + NLS-H1 (1.5μg/plate) + MAT-H1 (1.5μg/plate)<sup>(3,4)</sup><sub>(CB rare)</sub>
- 11> DNA + Lipo " " + NLS-H1 (3μg/plate)<sup>(6,8)</sup>
- 12> DNA + Lipo " " + NLS-H1 (4.5μg/plate)<sup>(10,2)</sup>
- 13> [DNA + NLS-H1 (1.5μg)] + DOPE (60λ/plate)
- 14> [DNA + NLS-H1 (1.5μg) + MAT-H1 (0.5μg)] + DOPE (60λ/plate)
- 15> [DOPE + NLS-H1 (1.5μg)] + DNA (5μg/plate)
- 16> [DOPE + NLS-H1 (1.5μg) + MAT-H1 (0.5μg)] + DNA.
- 17> DNA 5ug/plate + DOPE 60λ/plate

## 10 Day Old Myotubes -

- 1> DNA 6ug/plate) + Lipo (2.5λ/plate)
- 2> DNA " " + " " + NLS-H1 (1.5μg/plate)
- 3> DNA " " + Lipo " " + NLS-H1 " " + MAT-H1 (0.5μg)

# for all samples except 23-32

- Add DNA (10 μg/2ml media) in 100 μl Tris 8.5 + Protein  
↓ 15' rt

Add Lipofectin

↓ 80' rt

Add to 1.5 ml on each  $\phi$  35 mm dish

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5:28

MEAS TIME(s) 31.0

SAMPLE	RLU
1	H <sub>2</sub> O Blk
2	543
3	9204 H Lucifer
4	87500 5A Lucifer
5	> 5 μg/plate pBSRLUX
6	2385
7	44422 > DNA + 2.5 μg/plate Lipofectin
8	227053 > DNA + Lipo
9	415636 1.5μg/plate
10	16825 > DNA + Lipo + NLS-H1 (0.5μg/plate)
11	7391 (2.5)
12	763602 > DNA + Lipo + NLS-H1 (1.5μg/plate)
13	727847
14	56090 > DNA + Lipo + NLS-H1 + MAT-H1 (0.5μg/plate)
15	10582 (0.5μg/plate) (0.5μg/plate)
16	41314 > DNA + Lipo + NLS-H1 + MAT-H1 (0.5μg)
17	12334 (1.5μg) (1.5μg/plate)
18	18100 > DNA + Lipo + NLS-H1 + MAT-H1 (0.5μg)
19	31813 (1.5μg)
20	20410 > DNA + Lipo + NLS-H1 + MAT-H1 (1.5μg)
21	30253 (1.5μg) (1.5μg)
22	2381512 > DNA + Lipo + NLS-H1
23	939243 (3μg/plate)
24	5892126 > DNA + Lipo + NLS-H1
25	3102207 (4.5μg/plate)
26	182370 [DNA + NLS-H1] + DOPE 60 μg/plate
27	331964 (1.5μg) (1.5μg)
28	225842 [DNA + NLS-H1 + MAT-H1] + DOPE
29	248196 (1.5μg) (1.5μg)
30	132754 [DOPE + NLS-H1] + DNA
31	119066 (1.5μg)
32	406366 [DOPE + NLS-H1 + MAT-H1] + DNA
33	240774 [DNA + [DOPE] + NLS-H1] + DNA
34	87183 (1.5μg)
35	18896 (1.5μg)

\* MAT-H1 - purified via  
Ni-NTA agarose and then  
Cibacron Blue agarose

- loaded onto C B column at 150 mM NaCl + eluted off with 50 mM increasing NaCl step gradient
- checked on 12% SDS-PAGE
- concentrated single band containing (MAT-H1) elution
- contained 10  $\mu$ g protein (conc)

- Results - Appear that CB purified protein inhibits transfectability

\* Does it bind or retard DNA in a gel-shift assay

6/7/94

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## PS/PE expt #2

- 2 > PBSRLYKU<sup>(sm)</sup> + PS2.5<sup>182</sup> + NLS-1+1 (2ug) 8λ (300 DNA/prof) + (200 Liposome)
- 3 > DNA + PS2.5<sup>182</sup> + .. (4ug) 16λ
- 4 > DNA + PS2.5<sup>182</sup> + NLS 111 (6ug) 24λ
- 5 > DNA + PS5<sup>182</sup> + .. (2ug) 8λ
- 6 > DNA + PS5<sup>182</sup> + .. (4ug) 16λ
- 7 > DNA + PS5<sup>182</sup> + .. (6ug) 24λ
- 8 > DNA + PS10<sup>182</sup> + .. (2ug) 8λ
- 9 > DNA + PS10<sup>182</sup> + .. (4ug) 16λ
- 10 > DNA + PS10<sup>182</sup> + .. (6ug) 24λ
- 11 > DNA + PS10<sup>182</sup> + .. (2ug) 8λ
- 12 > DNA + PS10<sup>182</sup> + .. (4ug) 16λ
- 13 > DNA + PS10<sup>182</sup> + .. (6ug) 24λ
- 14 > DNA + PS2.5<sup>362</sup> + .. (2ug) 8λ
- 15 > DNA + PS2.5<sup>362</sup> + .. (4ug) 16λ
- 16 > DNA + PS2.5<sup>362</sup> + .. (6ug) 24λ
- 17 > DNA + PS2.5<sup>362</sup> + .. (2ug) 8λ
- 18 > DNA + PS2.5<sup>362</sup> + .. (4ug) 16λ
- 19 > DNA + PS2.5<sup>362</sup> + .. (6ug) 24λ
- 20 > DNA + PS2.5<sup>362</sup> + .. (2ug) 8λ
- 21 > DNA + PS2.5<sup>362</sup> + .. (4ug) 16λ
- 22 > DNA + PS2.5<sup>362</sup> + .. (6ug) 24λ
- 23 > DNA + PS5<sup>362</sup> + .. (2ug) 8λ
- 24 > DNA + PS5<sup>362</sup> + .. (4ug) 16λ
- 25 > DNA + PS5<sup>362</sup> + .. (6ug) 24λ
- 26 > DNA + PS10<sup>362</sup> + .. (2ug) 8λ
- 27 > DNA + PS10<sup>362</sup> + .. (4ug) 16λ
- 28 > DNA + PS10<sup>362</sup> + .. (6ug) 24λ
- 29 > DNA + PS10<sup>362</sup> + .. (2ug) 8λ
- 30 > DNA + PS10<sup>362</sup> + .. (4ug) 16λ
- 31 > DNA + PS10<sup>362</sup> + .. (6ug) 24λ
- 32 > DNA + ~~Lipofectin~~ 24λ (300 DNA) + (300 Liposome)
- 33 > DNA + ~~Lipofectin~~ 24λ (300 DNA) + (300 Liposome)

## Protocol

- Add DNA + protein (300d optimum) 15' at rt
- Add Liposomes in (200d optimum) 15' at rt
- wash cells 1X in optimum
- Add complex to 2ml optimum on cells
- change media after 3-4 hr
- incubate at 37°C for ~48 hrs
- harvest cells
- lux assay

• 3T3

- RLU  
 1191 >DNA + PS<sub>2.5</sub> + NLS-H1  
 466 (18A) (2ug)
- 3 66525 >DNA + PS<sub>2.5</sub> + NLS-H1  
 4 107400 18A 4ug
- 5 2181385 >DNA + PS<sub>2.5</sub> + NLS-H1  
 6 1997461 18A (6ug)
- 7 485 >DNA + PS<sub>5</sub> + NLS-H1  
 8 487 18A (2ug)
- 9 843961 >DNA + PS<sub>5</sub> + NLS-H1  
 10 799857 18A (4ug)
- 11 2443514 >DNA + PS<sub>5</sub> + NLS-H1  
 12 1974928 18A (6ug)
- 13 686 >DNA + PS<sub>10</sub> + NLS-H1  
 14 665 18A (2ug)
- 15 424442 >DNA + PS<sub>10</sub> + NLS-H1  
 16 31953 18A (4ug)
- 17 1930695 >DNA + PS<sub>10</sub> + NLS-H1  
 18 1779958 18A (6ug)
- 19 366265 >DNA + PS<sub>2.5</sub> + NLS-H1  
 20 649356 (36A) (4ug)
- 21 2776187 >DNA + PS<sub>2.5</sub> + NLS-H1  
 22 1149081 (36A) (6ug)
- 23 1596987 >DNA + PS<sub>5</sub> + NLS-H1  
 24 1792688 (36) (4ug)
- 25 2789949 >DNA + PS<sub>5</sub> + NLS-H1  
 26 3353918 (36A) (6ug)
- 27 270468 >DNA + PS<sub>10</sub> + NLS-H1  
 28 236696 (36A) (4ug)
- 29 2482591 >DNA + PS<sub>10</sub> + NLS-H1  
 30 2774275 (36A) (6ug)
- 31 2890371 >DNA + PS<sub>10</sub> + NLS-H1  
 32 2966735 (36A) (9ug)
- 33 273358 >DNA + PS<sub>10</sub> + NLS-H1  
 34 285150 (36A) (6ug)